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# Thermal Denaturation Study on The Binding of A Methylene Blue Derivative to A Triple Helix Forming Oligonucleotide

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**Abstract**

Photoactive methylene blue (MB) is a cationic planar chromophore which is known to interact with double helical and triple helical DNA. UV spectroscopy have been employed to investigate the interaction between this dye and various DNA sequences, thus complementing the indirect data from LD, CD and/or fluorescence spectroscopic data available up to now. Covalent attachment of the MB to the DNA through a flexible heptamethylene linker has been accomplished in several steps, enabling the defined positioning of the dye moiety at specific sites of the DNA strands.

UV thermal melting experiments indicate no significant stabilization/destabilization, demonstrated that MB interacts with all triplex-forming DNA sequences studied here, albeit in different binding modes and strengths. In contrast, for sequences with the duplex overhang moved to the other side of the construct and with the MB moiety facing the terminal T-A·T triplex tract, imino resonances were noticeably sharper compared to the identical triplex sequence without MB. The corresponding triple helix-MB conjugate was stabilized through the dye-DNA interactions by up to 10°C as indicated by the UV melting data.

Thus, under our experimental conditions (100 mM NaCl, pH=5) MB does not show any particular base specificity towards alternating CG base pairs as previously concluded by some other investigators through indirect methods. Moreover, a tract of three contiguous T-A·T triplets seems to be a preferable interaction site for MB, resulting in a significant stabilization of the triplex structures upon binding of MB ($\Delta T_m$=6.1°C). Stabilization further increases, when the two Watson-Crick strands of the T-A·T tract are stabilized by a

connecting loop ($\Delta T_m$=10°C). Furthermore, noticeable improvements of the 1D $^1$H NMR proton signals (sharper and better resolved lines) are observed upon MB attachment, confirming the specific stabilization of triplex structures through MB interactions.

**Keywords:** UV Thermal Denaturation, Methylene Blue, DNA-ligand interaction

## INTRODUCTION

Triple helix formation has also been found to offer the basis for numerous site-specific manipulations on duplex DNA. Appropriately designed third strand oligonucleotides targeting a duplex to form a triplex structure might be used to control gene expression by repressing the transcription step, to modulate the sequence-specifity of DNA-binding drugs or to selectively alter the sites of protein activity. Thus, many efforts have been dedicated to modify the third strand for particular purposes, e.g., by covalent attachment of certain ligands to the third oligonucleotide strand. Sequence-specific damage and cleavage of DNA strands employing photosensitizers or/and intercalators tethered to third strand oligonucleotides have also been reported.[4-9]

Methylene blue (MB), a phenothiazinium dye, is a promising ligand that has been shown to interact with DNA and is able to cause a strand cleavage after photoactivation by generating singlet oxygen.[10-16] This photosensitization process on MB might find its application as a noninvasive therapeutic tool in modern photomedicine, thus making this compound to be of big interest.[10,17]

In this research we report on an experimental study employing UV melting experiments as well as 1D $^1$H NMR to initialize the binding mode characterization between MB and DNA and also to gain preliminary information about the the formation, stability and structure of the complex. In order to specify the preferred MB binding location, which in turn may lead to its potential use in the site-directed damaging of DNA targets, we have previously synthesized a MB-oligonucleotide conjugate where a MB derivative was covalently coupled to a 3'-aminoalkyl-modified DNA oligonucleotide third strand by a flexible hydrocarbon linker (Fig. 1). Such a system, we hope, will not only reduce problems due to various stoichiometries, but also will avoid the coexistence of different interactions and lead to the formation of a complex with a better defined structure. The resulting oligonucleotide-dye conjugate was added to a complementary DNA double helix and formed triple helices were characterized by UV and NMR spectroscopic methods.